IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

DAVID HUGH JONES

Group Art Unit: 1656

Examiner: Noakes, Suzanne M.

Serial No.: 10/595,954

Filed: May 22, 2006

For: PURIFICATION METHOD FOR A RECOMBINANT GLUCOSE BINDING PROTEIN

Attorney Docket No.: KIST0101PUSA

DECLARATION OF DAVID HUGH JONES UNDER 37 C.F.R. § 1.132

Commissioner for Patents
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

I, David Hugh Jones, do hereby declare and state as follows:

1. After my Bachelors degree in 1978 from the University of Cambridge with Upper Second Class Honours in Biochemistry, I pursued graduate work in Biochemistry and obtained my Ph.D. from the University of Cambridge in 1983.

2. Since 1981, I have held various academic positions. From 1981 to 1984, I was a Tucker-Price Research Fellow in Biochemistry (Enzymology), Girton College and Department of Biochemistry, University of Cambridge. From 1984 to 1986, I was a Post-Doctoral Research Assistant (Protein Engineering), Department of Chemistry, Imperial College of Science and Technology, University of London. From 1998 to 2006, I was a Senior Lecturer and member of Molecular Biology Research Group, School of Biological Sciences, University of Wales, Swansea. From 2006, I have been a Senior Lecturer, School of Medicine, University of Wales Swansea.

3. From 2008, I have been a member of the Welsh Food Advisory Committee appointed by the Welsh Assembly Government to advise the UK Food Standards Agency in Wales. I am also a member of the following scientific organizations: the Protein Society (USA), the Biochemical Society (UK), the Society for General Microbiology (UK), and the Royal Society of chemistry (UK).

4. I am the author of numerous peer-reviewed scientific publications. A list of these publications and patent publications is attached hereto.

5. I am the inventor of the claimed subject matter of U.S. Application Serial No. 10/595,954, hereinafter the "Application", and am familiar with the content of the Application and the Office Action dated January 22, 2009 received from the United States Patent and Trademark Office, hereinafter the "Office Action."

6. I understand claims 1-4, 14-16, and 20-21 of the Application stand rejected under 35 U.S.C. § 102(b) as being unpatentable over *Min et al.* (EMBO J., 1992, 11(4):1303-1307; hereinafter *Min*).

7. I was the senior author of *Min* and in fact, *Min* was provided as a reference when the Application was filed. *Min* does not recognize the problems associated with glycogen, namely that glycogen complexes with recombinant mature Concanavalin A (Con A) proteins and negatively affects Con A recovery. *Min* makes no mention of the issues surrounding purification of protein product in relation to glycogen, nor does *Min* demonstrate the protein product is or should be substantially free of glycogen and other impurities. The gel photograph in Figure 2 of *Min* merely indicates that the protein product as dissolved in the gel binds no other proteins of revealable sizes, however, the gel photograph does not speak to whether the protein product is contaminated with non-protein contaminants such as glycogen and nucleic acids. While being possibly able to dissolve glycogen, *Min*'s buffer is not used to discriminate glycogen from the protein product. On the contrary, reducing glycogen content

in the insoluble fraction of the lysate in favor of retaining the active Con A protein is the specific solution provided only by the claimed invention.

8. Moreover, *Min* is directed to recombinant pro-Con A, which is fundamentally different from the recombinant mature Con A recited in the claimed invention. For instance, and as disclosed in *Min*, the mature form of Con A is unique in being circularly permuted in amino acid sequence relative to its own precursor (pro-Con A) and other legume lectins, while Pro-ConA is not so permuted relative to other legume lectins.

9. I have compared the minimum levels of contaminants including glycogen in a recombinant Con A sample treated substantially according to the method of *Min*, relative to the contaminant levels in a comparison sample treated according to the method of the Application, with corresponding results shown in Table I below. Since actual samples and chemicals used in *Min*, a 17-year-old publication, are no longer available, in conducting the experiment, I have used constructs and chemicals currently obtainable that are of similar characteristics. In particular, I have produced a construct in a pET26b(+) vector in *Escherichia coli* BL21(DE3) cells cultured in LB medium with 1% w/v glucose. Methods for measuring non-dialysable carbohydrate including glycogen by phenol-sulphuric acid assay, for measuring glycogen by the enzymatic hydrolysis/coupled assay, and for measuring nucleic acid by the Warburg-Christian absorbance ratio assay, are described in the Application as originally filed, from line 17 on page 31 to line 14 on page 32, or [0126]-[0127] of the Application as published.

10. As shown in Table I, the contaminant levels of the Con A sample according to *Min*'s method are significantly higher than the contaminant levels of the Con A sample according to the Applicant's method.

Table I

Contaminants	using Min's method	using Applicant's method
non-dialysable carbohydrates including glycogen	2.60 % w/w protein	0.33 ± 0.03 % w/w protein
glycogen	1.00 % w/w protein	0.03 ± 0.01 % w/w protein
nucleic acids	2.80 % w/w protein	0.03 ± 0.02 % w/w protein

11. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the Application and any patent issued therein.

Signed: N. H. Jones Date: May 21st, 2009

List of Publications of David Hugh Jones

Jones, D.H. & Northcote, D.H. (1981) "Induction by hormones of phenylalanine ammonia-lyase in bean cell suspension culture: inhibition and superinduction by actinomycin D" *Eur.J.Biochem.* **116**, 117-125.

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Min, W., Dunn, A.J. & Jones, D.H. (1992) "Non-glycosylated recombinant pro-Concanavalin A is active without polypeptide cleavage" *EMBO J.* **11**, 1303-1307.

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Jones, D.H., Min, W., Dincturk, H.B., Dunn, A.J., Williams, R.P. & Li, M. (1996) "Recombinant Concanavalin A and precursors used to investigate its extraordinary post-translation processing " in *Lectins: Biology, Biochemistry, Clinical Biochemistry*, Van Driessche, E. Rouge, P., Beeckmans, S. & Bog-Hansen, T.C. (eds.) vol **11**. pp. 70-73. Textop, Denmark [ISBN 87-984583-2-9]

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Jones, D. H. (2007) "Purification method for a recombinant glucose binding protein", **PATENT APPLICATION** (United States Patent Application), Publication Number: US 2007/0142624 A1 (21 June 2007).